(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 708 178 A1

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
24.04.1996 Bulletin 1996/17

(21) Application number: **94402353.0**

(22) Date of filing: **19.10.1994**

(51) Int. Cl.[6]: **C12N 15/12**, C07K 14/47, C12N 15/11, C12Q 1/68, C12N 5/10, C07K 16/18

(84) Designated Contracting States:
FR

(71) Applicant: **INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE (INSERM)**
F-75654 Paris Cédex 13 (FR)

(72) Inventors:
- **Melki, Judith**
  75015 Paris (FR)
- **Munnich, Arnold**
  75006 Paris (FR)

(74) Representative: **Desaix, Anne et al**
Ernest Gutmann - Yves Plasseraud S.A.
3, rue Chauveau-Lagarde
F-75008 Paris (FR)

Remarks:
The applicant has subsequently filed a sequence listing and declared, that it includes no new matter.

(54) Survival motor neuron (SMN) gene: a gene for spinal muscular atrophy

(57) The present invention relates to the discovery of a survival motor-neuron gene or SMN gene which is a chromosome 5-SMA (Spinal Muscular Atrophy) determining gene. The present invention further relates to the nucleotide sequence encoding the SMN gene and corresponding amino acid sequence, a vector containing the gene encoding the SMN protein or a DNA sequence corresponding to the gene and transformant strains containing the SMN gene or a DNA sequence corresponding to the gene.

The present invention also relates to means and methods for detecting motor neuron diseases having symptoms of muscular weakness with or without sensory changes such as amytrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), primary lateral sclerosis (PLS) and the like.

EP 0 708 178 A1

Printed by Rank Xerox (UK) Business Services
2.11.3/3.4

MAMSSGGSGGGVPEQEDSVLFRRGTGQSDDSDIWDDTALIKAYDKAVAS
FKHALKNGDICETSGKPKTTPKRKPAKKNKSQKKNTAASLQQWKVGDKCSAIWSEDGCIY
PATIASIDFKRETCVVVYTGYGNREEQNLSDLLSPICEVANNIEQNAQENENESQVSTDE
SENSRSPGNKSDNIKPKSAPWNSFLPPPPPMPGPRLGPGKPGLKFNGPPPPPPPPPPHLL
SCWLPPFPSGPPIIPPPPPICPDSLDDADALGSMLISWYMSGYHTGYYMGFRQNQKEGRC
SHSLN

FIGURE 1

CGGGGCCCCACGCTGCGCACCCGCGGGTTTGCTATGGCGATGAGCAGCGGCGGCAGTGGT
GGCGGCGTCCCGGAGCAGGAGGATTCCGTGCTGTTCCGGCGCGGCACAGGCCAGAGCGAT
GATTCTGACATTTGGGATGATACAGCACTGATAAAAGCATATGATAAAGCTGTGGCTTCA
TTTAAGCATGCTCTAAAGAATGGTGACATTTGTGAAACTTCGGGTAAACCAAAAACCACA
CCTAAAAGAAAACCTGCTAAGAAGAATAAAAGCCAAAAGAAGAATACTGCAGCTTCCTTA
CAACAGTGGAAAGTTGGGGACAAATGTTCTGCCATTTGGTCAGAAGACGGTTGCATTTAC
CCAGCTACCATTGCTTCAATTGATTTTAAGAGAGAAACCTGTGTTGTGGTTTACACTGGA
TATGGAAATAGAGAGGAGCAAAATCTGTCCGATCTACTTTCCCCAATCTGTGAAGTAGCT
AATAATATAGAACAGAATGCTCAAGAGAATGAAAATGAAAGCCAAGTTTCAACAGATGAA
AGTGAGAACTCCAGGTCTCCTGGAAATAAATCAGATAACATCAAGCCCAAATCTGCTCCA
TGGAACCCCTTTCTCCCTCCACCACCCCCCATGCCAGGGCCAAGACTGGGACCAGGAAAG
CCAGGTCTAAAATTCAATGGCCCACCACCGCCACCGCCACCACCACCACCCCACTTACTA
TCATGCTGGCTGCCTCCATTTCCTTCTGGACCACCAATAATTCCCCCACCACCTCCCATA
TGTCCAGATTCTCTTGATGATGCTGATGCTTTGGGAAGTATGTTAATTTCATGGTACATG
AGTGGCTATCATACTGGCTATTATATGGGTTTTAGACAAAATCAAAAAGAAGGAAGGTGC
TCACATTCCTTAAATTAAGGAGAAATGCTGGCATAGAGCAGCACTAAATGACACCACTAA
AGAAACGATCAGACAGATCTGGAATGTGAAGCGTTATAGAAGATAACTGGCCTCATTTCT
TCAAAATATCAAGTGTTGGGAAAGAAAAAAGGAAGTGGAATGGGTAACTCTTCTTGATTA
AAAGTTATGTAATAACCAAATGCAATGTGAAATATTTTACTGGACTCTTTTGAAAAACCA
TCTGTAAAAGACTGAGGTGGGGGTGGGAGGCCAGCACGGTGGTGAGGCAGTTGAGAAAAT
TTGAATGTGGATTAGATTTTGAATGATATTGGATAATTATTGGTAATTTTATGGCCTGTG
AGAAGGGTGTTGTAGTTTATAAAAGACTGTCTTAATTTGCATACTTAAGCATTTAGGAAT
GAAGTGTTAGAGTGTCTTAAAATGTTTCAAATGGTTTAACAAAATGTATGTGAGGCGTAT
GTGGCAAAATGTTACAGAATCTAACTGGTGGACATGGCTGTTCATTGTACTGTTTTTTTC
TATCTTCTATATGTTTAAAAGTATATAATAAAAATATTTAATTTTTTTTTAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAA

FIGURE 2A

AATTTTTTAAATTTTTTTGTAGAGACAGGGTCTCATTATGTTGCCCAGGGTGGTGTCAAGCTCCA
GGTCTCAAGTGATCCCCCTACCTCCGCCTCCCAAAGTTGTGGGATTGTAGGCATGAGCCACTG
CAAGAAAACCTTAACTGCAGCCTAATAATTGTTTTCTTTGGGATAACTTTTAAAGTACATTAA
AAGACTATCAACTTAATTTCTGATCATATTTTGTTGAATAAAATAAGTAAAATGTCTTGTGAA
CAAAATGCTTTTTTAACATCCATATAAAGCTATCTATATATAGCTATCTATATCTATATAGCTA
TTTTTTTTAACTTCCTTTTATTTTCCTTACAG*GGTTTCAGACAAAATCAAAAAGAAGGAAGG
TGCTCACATTCCTTAAATTAAGGA*GTAAGTCTGCCAGCATTATGAAAGTGAATCTTACTTTT
GTAAAACTTTATGGTTTGTGGAAAACAAATGTTTTTGAACAGTTAAAAAGTTCAGATGTTAGA
AAGTTGAAAGGTTAATGTAAAACAATCAATATTAAAGAATTTTGATGCCAAAACTATTAGATA
AAAGGTTAATCTACATCCCTACTAGAATTCTCATACTTAACTGGTTGGTTGTGTGGAAGAAAC
ATACTTTCACAATAAAGAGCTTTAGGATATGATGCCATTTTATATCACTAGTAGGCAGACCAG
CAGACTTTTTTTTATTGTGATATGGGATAACCTAGGCATACTGCACTGTACACTCTGACATAT
GAAGTGCTCTACTCAAGTTTAACTGGTGTCCACAGAGGACATGGTTTAACTGGAATTCGTCAA
GCCTCTGGTTCTAATTTCTCATTTGCAG*GAAATGCTGGCATAGAGCAGCACTAAATGACACC
ACTAAAGAAACGATCAGACAGATCTGCAATGTGAAGCGTTATAGAAGATAACTGGCCTCATTT
CTTCAAAATATCAAGTGTTGGGAAAGAAAAAAGGAAGTGGAATGGGTAACTCTTCTTGATTA
AAAGTTATGTAATAACCAAATGCAATGTGAAATATTTTACTGGACTCTTTTGAAAAAC
CATCTGTAAAAGACTGGGGTGGGGGTGGGAGGCCAGCACGGTGGTGAGGCAGTTGAGAAAA
TTTGAATGTGGATTAGATTTTGAATGATATTGGATAATTATTGGTAATTTTATGGCCTGT
GAGAAGGGTGTTGTAGTTTATAAAAGACTGTCTTAATTTGCATACTTAAGCATTTAGG
AATGAAGTGTTAGAGTGTCTTAAAATGTTTCAAATGGTTTAACAAAATGTATGTGAGGCGT
ATGTGGCAAAATGTTACAGAATCTAACTGGTGGACATGGCTGTTCATTGTACTGTTTTTT
TCTATCTTCTATATGTTTAAAAGTATATAATAAAAATATTTAATTT

FIGURE 2B

1
CGGGGCCCCACGCTGCGCATCCGCGGGTTTGCTATGGCGATGAGCAGCGGCGGCAGTGGT
2
GGCGGCGTCCCGGAGCAGGAGGATTCCGTGCTGTTCCGGCGCGGCACAGGCCAG*AGCGAT
GATTCTGACATTTGGGATGATACAGCACTGATAAAAGCATATGATAAAGCTGTGGCTTCA
TTTAAGCATGCTCTAAAGAATGGTGACATTTGTGAAACTTCGGGTAAACCAAAAACCACA
CCTAAAAGAAAACCTGCTAAGAAGAATAAAAGCCAAAAGAAGAATACTGCAGCTTCCTTA
3
CAACAG*TGGAAAGTTGGGGACAAATGTTCTGCCATTTGGTCAGAAGACGGTTGCATTTAC
CCAGCTACCATTGCTTCAATTGATTTTAAGAGAGAAACCTGTGTTGTGGTTTACACTGGA
TATGGAAATAGAGAGGAGCAAAATCTGTCCGATCTACTTTCCCCAATCTGTGAAGTAGCT
4
AATAATATAGAACAGAATGCTCAAGAG*AATGAAAATGAAAGCCAAGTTTCAACAGATGAA
AGTGAGAACTCCAGGTCTCCTGGAAATAAATCAGATAACATCAAGCCCAAATCTGCTCCA
TGGAACTCTTTTCTCCCTCCACCACCCCCCATGCCAGGGCCAAGACTGGGACCAGGAAAG
5
*CCAGGTCTAAAATTCAATGGCCCACCACCGCCACCGCCACCACCACCACCCCACTTACTA
6
TCATGCTGGCTGCCTCCATTTCCTTCTGGACCACCA*ATAATTCCCCCACCACCTCCCATA
TGTCCAGATTCTCTTGATGATGCTGATGCTTTGGGAAGTATGTTAATTTCATGGTACATG
7
AGTGGCTATCATACTGGCTATTATATG*GGTTTCAGACAAAATCAAAAAGAAGGAAGGTGC
8
TCACATTCCTTAAATTAAGGA*GAAATGCTGGCATAGAGCAGCACTAAATGACACCACTAA
AGAAACGATCAGACAGATCTGGAATGTGAAGCGTTATAGAAGATAACTGGCCTCATTTCT
TCAAAATATCAAGTGTTGGGAAAGAAAAAAGGAAGTGGAATGGGTAACTCTTCTTGATTA
AAAGTTATGTAATAACCAAATGCAATGTGAAATATTTTACTGGACTCTTTTGAAAAAC
CATCTGTAAAAGACTGGGGTGGGGGTGGGACGCCAGCACGGTGGTGAGGCAGTTGAGAAAA
TTTGAATGTGGATTAGATTTTGAATGATATTGGATAATTATTGGTAATTTTATGGCCTGT
GAGAAGGGTGTTGTAGTTTATAAAAGACTGTCTTAATTTGCATACTTAAGCATTTAGG
AATGAAGTGTTAGAGTGTCTTAAAATGTTTCAAATGGTTTAACAAAATGTATGTGAGGCGT
ATGTGGCAAAATGTTACAGAATCTAACTGGTGGACATGGCTGTTCATTGTACTGTTTTTT
TCTATCTTCTATATGTTTAAAAGTATATAATAAAAATATTTAATTTTTTTTTAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

FIGURE 3A

AATTTTTAAATTTTTTGTAGAGACAGGGTCTCATTATGTTGCCCAGGGTGGTGTCAAGCTCCA
GGTCTCAAGTGATCCCCCTACCTCCGCCTCCCAAAGTTGTGGGATTGTAGGCATGAGCCACTG
CAAGAAAACCTTAACTGCAGCCTAATAATTGTTTTCTTTGGGATAACTTTTAAAGTACATTAA
AAGACTATCAACTTAATTTCTGATCATATTTTGTTGAATAAAATAAGTAAAATGTCTTGTGAA
CAAAATGCTTTTTAACATCCATATAAAGCTATCTATATATAGCTATCTATGTCTATATAGCTA
TTTTTTTTAACTTCCTTTTATTTTCCTTACAG*GGTTTCAGACAAAATCAAAAAGAAGGAAGG
TGCTCACATTCCTTAAATTAAGGA*GTAAGTCTGCCAGCATTATGAAAGTGAATCTTACTTTT
GTAAAACTTTATGGTTTGTGGAAAACAAATGTTTTTGAACAGTTAAAAAGTTCAGATGTTAAA
AAGTTGAAAGGTTAATGTAAAACAATCAATATTAAAGAATTTTGATGCCAAAACTATTAGATA
AAAGGTTAATCTACATCCCTACTAGAATTCTCATACTTAACTGGTTGGTTATGTGGAAGAAAC
ATACTTTCACAATAAAGAGCTTTAGGATATGATGCCATTTTATATCACTAGTAGGCAGACCAG
CAGACTTTTTTTTATTGTGATATGGGATAACCTAGGCATACTGCACTGTACACTCTGACATAT
GAAGTGCTCTAGTCAAGTTTAACTGGTGTCCACAGAGGACATGGTTTAACTGGAATTCGTCAA
GCCTCTGGTTCTAATTTCTCATTTGCAG*GAAATGCTGGCATAGAGCAGCACTAAATGACACC
ACTAAAGAAACGATCAGACAGATCTGGAATGTGAAGCGTTATAGAAGATAACTGGCCTCATTT
CTTCAAAATATCAAGTGTTGGGAAAGAAAAAAGGAAGTGGAATGGGTAACTCTTCTTGATTA
AAAGTTATGTAATAACCAAATGCAATGTGAAATATTTTACTGGACTCTTTTGAAAAAC
CATCTGTAAAAGACTGGGGTGGGGGTGGGAGGCCAGCACGGTGGTGAGGCAGTTGAGAAAA
TTTGAATGTGGATTAGATTTTGAATGATATTGGATAATTATTGGTAATTTTATGGCCTGT
GAGAAGGGTGTTGTAGTTTATAAAAGACTGTCTTAATTTGCATACTTAAGCATTTAGG
AATGAAGTGTTAGAGTGTCTTAAAATGTTTCAAATGGTTTAACAAAATGTATGTGAGGCGT
ATGTGGCAAAATGTTACAGAATCTAACTGGTGGACATGGCTGTTCATTGTACTGTTTTTT
TCTATCTTCTATATGTTTAAAAGTATATAATAAAAATATTTAATTT

FIGURE 3B

C212

ACCTGANCCCAGANGGTCAAGGCTGCAGTGAGACGAGATTGCNCCACTGCCCTCC
ACCCTGGGTGATAAGAGTGGGACCCTGTNTCAAAACATACACACACACACACACA
CACACACACACACACACACACACACTCTCTCTCTCTCTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTCTCAAAAACACTTGGTCTGTTATTTTTNCGAAATTGTCAGTCAT
AGTTATCTGTTAGACCAAAGCTGNGTAAGNACATTTATTACATTGCCTCCTACAA
CTTCATCAGCTAATGTATTTGCTATATAGCAATTACATATNGGNATATATTATCT
TNAGGGGATGGCCANGTNATAAAACTGTCACTGAGGAAAGGA

C272

CCTCCCACCTNAGCCTCCCCAGTAGCTAGGACTATAGGCGTGCNCCACCAAGCTC
AGCTATTTTTNNTATTTAGTAGAGACGGGGTTTCGGCANGCTTAGGCCTCGTNTC
GAACTCCAGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTG
TGTGTAGATATTTATTCCCCCTCCCCCTTGGAAAAGTAAGTAAGCTCCTACTAGG
AATTTAAAACCTGCTTGATCTATATAAAGACAAACAAGGAAAGACAAACATGGGG
GCAGGAAGGAAGGCAGATC

AFM157xd10

TCGAGGTAGATTTGTATTATATCCCATGTACACACACACACACACACACACACAC
ACACACACACACAGACTTAATCTGTTTACAGAAATAAAAGGAATAAAATACCGTT
TCTACTATACACCAAAACTAGCCATCTTGAC

C161

CCCTGAGAAGGCTTCCTCCTGAGTATGCATAAACATTCACAGCTTGCATGCGTGT
GTGTGTGTGTGTGTGTGTGTATGTTTGCTTGCACTGTAAAAACAATTGCAACATC
AACAGAAATAAAAATTAAAGGAATAATTCTCCTCCGACTCTGCCGTTCCATCCAG
TGAAACTCTTCATTCTGGGGTAAAGTTCCTTCAGTTCTTTCATAGATAGGTATAT
ACTTCATAAGTCAAACAATCAGGCTGGGTGCAGTAGCTCATGCCTGTAATCCCAG
CCCTTTGGGAGGCCGAGCTGGGCAGATCGA

C171

TCCACCCGCCTTGGCCTCCCAAAGCNCTGGGATTACAGGCGTGACTGCCGCACCC
AGCTGTAAACTGGNTTNNTAATGGTAGATTTTNAGGTATTAACAATAGATAAAAA
GATACTTTTNGGCATACTGTGTATTGGGATGGGGTTAGAACAGGTGTNCTACCCA
AGACATTTACTTAAAATCGCCCTCGAAATGCTATGTGAGCTGTGTGTGTGTGTGT
GTGTGTGTGTGTATTAAGGAAAAGCATGAAAGTATTTATGCTTGATTTTTTTTTT
TNACTCATAGCTTCATAGTGGANCAGATACATAGTCTAAATCAAAATGTTTAAAC
TTTTTATGTCACTTGCTGTC

FIGURE 4

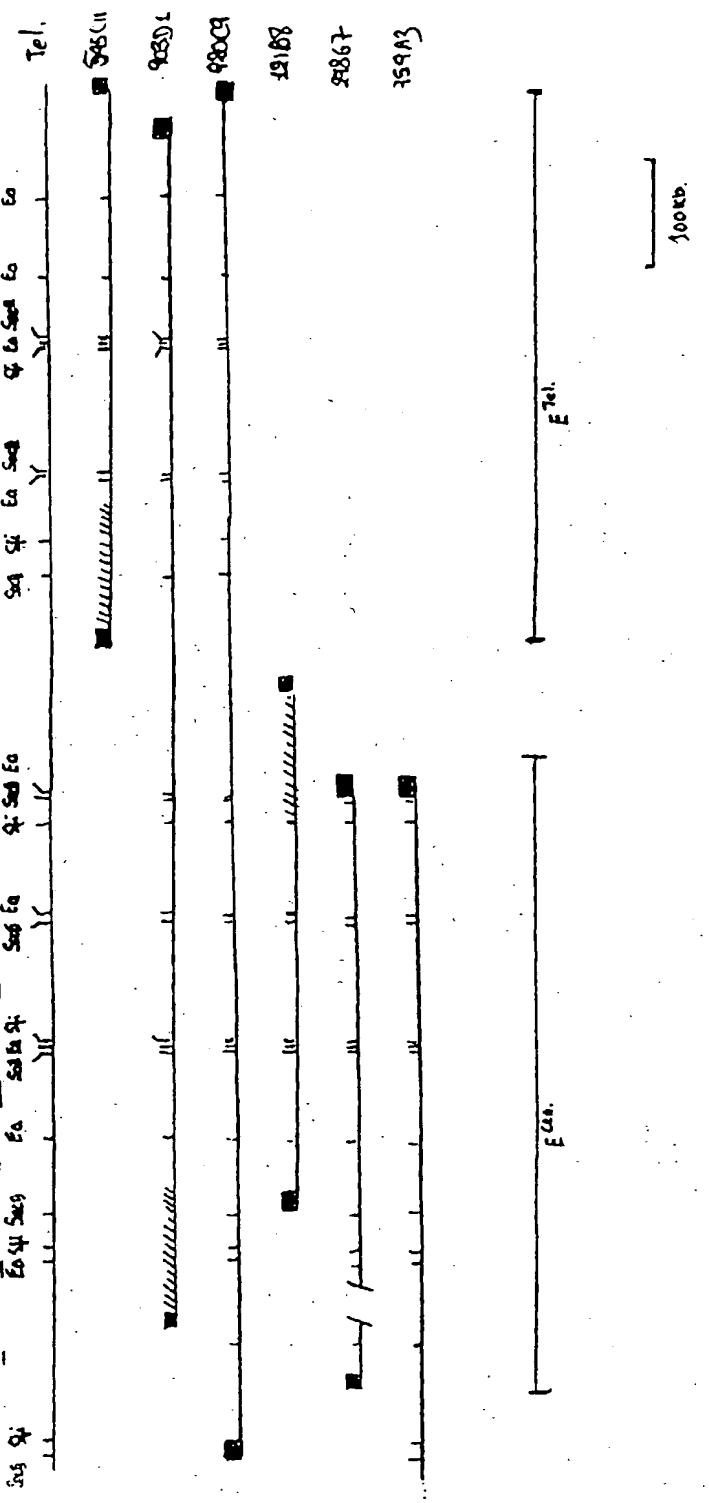
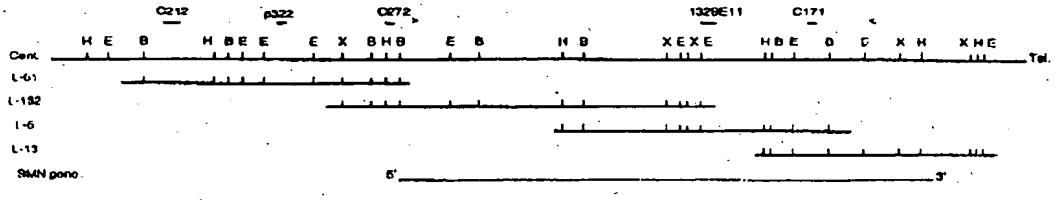


FIGURE 5

Restriction map of the 5q13 region. For EagI (Ea), SacII (SacII), SfiI (Sfi). Numbers under parenthesis indicate the restriction fragment declared by He3; Telomeric element ($E^{Tel.}$), Centromeric element ($E^{Cen.}$) Cen.(Cent.), Tel.(Tel.) Probes are indicated above the restriction map. YACs are below the restriction map.



Telomeric element (E^{Tel}) containing the survival motor-neuron gene (SMN gene). Genetic map shows polymorphic markers C212, C272 and C171. Physical map shows location and direction of transcription of SMN gene; phage clones used for assembling physical map. Restriction map for EcoRI(E), XbaI(X), HindIII(H), BglII(B), SacII(S) are shown. Cent. and Tel. indicate centromere and telomere respectively. The position of genomic rearrangements found in SMA patients are also indicated.

FIGURE 6

Gene dosage analysis of the 5q13 region with the 132SE11 plasmid cone in SMA type I patient. Total human DNA from SMA family was digested with HindIII for Southern blotting. Filter was consecutively hybridized with 132SE11 (A) and JK53 probes (B). A significant decrease in 132SE11 band intensity, which indicated the deletion, compared with their parents. F/Father, M/Mother, A/affected

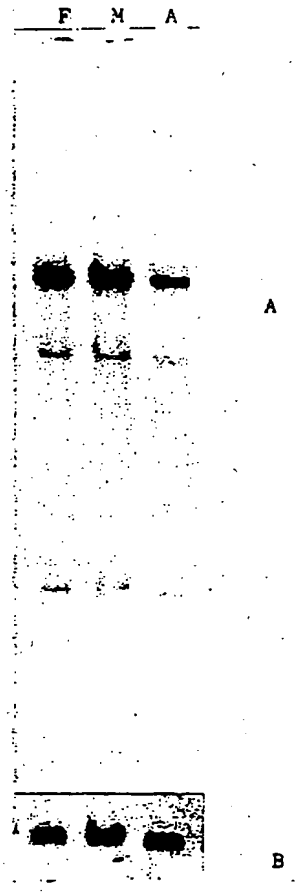


FIGURE 7

MAMSSGGSGGGVPEQEDSVLFRRGTGQSDDSDIWDDTALIKAYDKAVASFKHA
LKNGDICETSGKPKTTPKRKPAKKNKSQKKNTAASLQQWKVGDKCSAIWSEDG
CIYPATIASIDFKRETCVVVYTGYGNREEQNLSDLLSPICEVANNIEQNAQEN
ENESQVSTDESENSRSPGNKSDNIKPKSAPWNSFLPPPPPMPGPRLGPGKPGL
KFNGPPPPPPPPPPHLLSCWLPPFPSGPPIIPPPPPICPDSLDDADALGSMLI
SWYMSGYHTGYYM

FIGURE 8

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 94 40 2353

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	SEMINARS IN NEUROLOGY, vol.13, no.3, September 1993 pages 276 - 282 P. KLEYN AND T. GILLIAM 'Progress toward cloning of the gene responsible for childhood spinal muscular atrophy'	1-18, 22-27,33	C12N15/12 C07K14/47 C12N15/11 C12Q1/68 C12N5/10 C07K16/18
Y	* the whole document *	19-21, 28-32	

X	THE AMERICAN JOURNAL OF HUMAN GENETICS, vol.55, no.3, September 1994 page A31 C. DIDONATO ET AL 'Identification of strong allele association and candidate cDNAs for the spinal muscular atrophy gene'	1-18,22, 27,33	
Y	* abstract 153 *	19-21, 28-32	

X	THE AMERICAN JOURNAL OF HUMAN GENETICS, vol.55, no.3, September 1994 page A2691 B. ROSS ET AL 'Isolation of SMA candidate genes from a YAC contig by direct selection of cDNA from normalized cDNA libraries'	1-18,22, 27,33	TECHNICAL FIELDS SEARCHED (Int.Cl.6) C12N C12Q C07K
Y	* abstract 1573 *	19-21, 28-32	

Y	TRENDS IN BIOTECHNOLOGY., vol.5, no.4, 1987, CAMBRIDGE GB pages 107 - 111 D. KINGSBURY 'DNA probes in the diagnosis of genetic and infectious diseases' * the whole document *	19-21, 28-32	

	-/--		

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	21 March 1995	Van der Schaal, C

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 94 40 2353

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Y	WO-A-92 00386 (THE TRUSTEES OF COLOMBIA UNIVERSITY IN THE CITY OF NEW YORK) 9 January 1992 * the whole document * ---	19-21, 28-32	
A	THE AMERICAN JOURNAL OF HUMAN GENETICS, vol.55, no.3, September 1994 page A31 T. CARTER ET AL 'Identification of candidate genes in the spinal muscular atrophy gene region' * abstract 154 * -----	1-18,22, 27,33	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	21 March 1995	Van der Schaal, C

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)